

## Supplementary Text

### Explorations of additional candidate genes associated with root length

#### *A novel association between mean root length and At5g45120*

We identified an SNV on chromosome 5 (18,241,640 bp - SNP 5\_18241640) in the epistatic GWAS analysis through its interaction with SNV 3\_66596 (Table 2). Further analyses showed that this SNV was in LD with two missense SNVs and four synonymous SNVs in the gene At5g45120. The insertion mutant of At5g45120, however, showed wild-type root length (Figure 4), possibly due to the limitations of single-mutant analysis in validating epistatic interactions.

At5g45120 encodes a member of the little studied eukaryotic aspartyl protease family (Table 4) [1]. The two missense variants, I->T and D->A, were positioned 5 bp apart and were either adjacent to or within a ‘phosphorylation hotspot’, suggesting that post-translational modifications may be altered in some accessions [2,3]. Although mutating At5g45120 in a Col-0 genetic background did not affect mean root length, future exploration of this mutant or the minor allele in alternative genetic backgrounds may reveal whether At5g45120 affects root development.

#### *At3g01180 and At3g01185 are positional candidate genes for mean root length*

The SNV 3\_66596 was associated with mean root length in the epistatic GWAS through interactions with i) two SNVs at the *ATL5* locus (Table 2) and ii) a SNV on chromosome 5 (5\_18241640). The SNV resides in the gene At3g01185, a 148 amino acid protein of unknown function (Table 4) [4], where it results in a synonymous change. This SNV also resides in the adjacent promoter region of the gene At3g01180, which is highly accessible by DNase I [5]. As

this SNV is not in LD with any other SNVs in the 1001 Genomes re-sequencing data, it might be the causal SNV for the observed association, either by directly affecting the function of At3g01185 or by altering the expression of At3g01180.

We phenotyped the roots of an insertion mutant for At3g01185, but failed to find significant effects on root growth (Figure 4), implying that disruption of this gene alone is not sufficient to affect root development. Unfortunately, an insertion mutant for At3g01180 was not available. Further studies, including generating and phenotyping At3g01180 mutants, will be required to either validate or exclude one of these candidate genes.

#### *Epistatic interaction with a transposon-rich region on chromosome 1*

SNV 1\_17257526 interacted with the *NAC6* locus on chromosome 5. This SNV is located in the gypsy-like transposon At1g46624 and was not in LD with any other SNVs. The 20kb region surrounding the peak association encodes 16 transposable elements and many regulatory elements as identified by DNaseI-accessible chromatin [5]. How a polymorphism in this complex and potentially volatile region would affect root length by itself, or through epistatic interactions, is unclear and deserves further investigation.

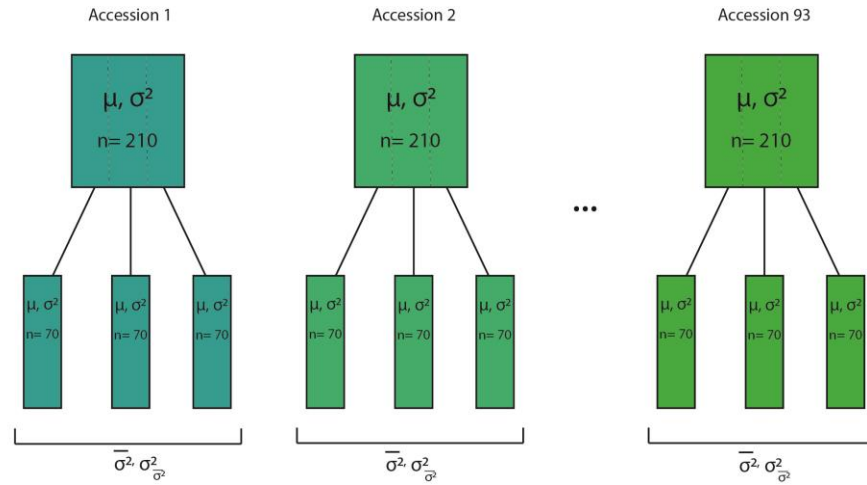
## **References**

1. Beers EP, Jones AM, Dickerman AW (2004) The S8 serine, C1A cysteine and A1 aspartic protease families in Arabidopsis. *Phytochemistry* 65: 43–58. Available: <http://www.sciencedirect.com/science/article/pii/S003194220300565X>. Accessed 5 December 2013.
2. Heazlewood JL, Durek P, Hummel J, Selbig J, Weckwerth W, et al. (2008) PhosPhAt: a database of phosphorylation sites in Arabidopsis thaliana and a plant-specific phosphorylation site predictor. *Nucleic Acids Res* 36: D1015–21. Available:

[http://nar.oxfordjournals.org/content/36/suppl\\_1/D1015.long](http://nar.oxfordjournals.org/content/36/suppl_1/D1015.long). Accessed 10 November 2013.

3. Durek P, Schmidt R, Heazlewood JL, Jones A, MacLean D, et al. (2010) PhosPhAt: the *Arabidopsis thaliana* phosphorylation site database. An update. *Nucleic Acids Res* 38: D828–34. Available: [http://nar.oxfordjournals.org/content/38/suppl\\_1/D828.long](http://nar.oxfordjournals.org/content/38/suppl_1/D828.long). Accessed 10 November 2013.
4. Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C, et al. (2012) The *Arabidopsis* Information Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Res* 40: D1202–D1210.
5. Sullivan A, Arsovski A, Lempe J, Bubb K, Weirauch M, et al. (2014) Mapping and dynamics of regulatory DNA and transcription factor networks in *A. thaliana*. *Cell Rep* In press.

## Supplemental Figures

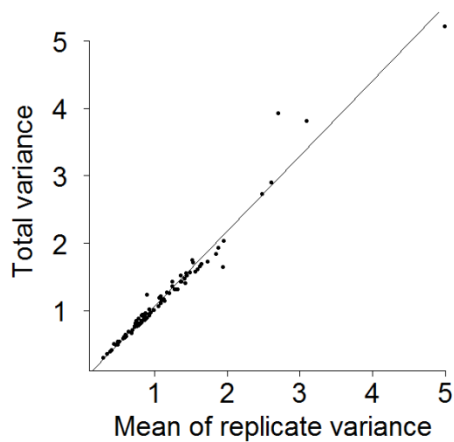


**Figure S1. Experimental design to increase accuracy of variance estimates.** For 93 accessions, a total of 210 seedlings were grown in three replicates of 70 individuals. For each replicate, the mean and variance was estimated. The mean and variance ( $\bar{\sigma}^2, \sigma^2_{\sigma^2}$ ) of the variance across three replicates was also calculated.

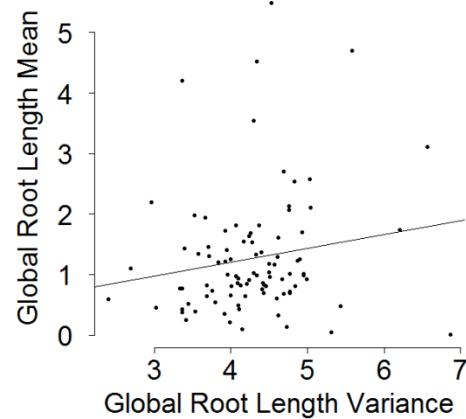
accession	stock number	accession	stock number	accession	stock number
Ag-0	CS22630	Kin-0	CS22654	Ren-11	CS22611
An-1	CS22626	Knox-10	CS22411	Rmx-A02	CS22568
Bay-0	CS22633	Knox-18	CS22567	Rmx-A180	CS22569
Bil-5	CS22578	Kondara	CS22651	RRS-10	CS22565
Bor-1	CS22590	Kz-1	CS22606	RRS-7	CS22564
Bor-4	CS22591	Kz-9	CS22607	Se-0	CS22646
Br-0	CS22628	Ler-1	CS22618	Shakdara	CS22652
Bur-0	CS22656	LL-0	CS22650	Sorbo	CS22653
C24	CS22620	Löv-1	CS22574	Spr1-2	CS22582
CIBC-17	CS22603	Löv-5	CS22575	Sq-1	CS22600
CIBC-5	CS22602	Lp2-2	CS22594	Sq-8	CS22601
Col-0	CS22625	Lp2-6	CS22595	Tamm-2	CS22604
N13	CS22491	Lz-0	CS22615	Tamm-27	CS22605
Ct-1	CS22639	Mr-0	CS22640	Ts-1	CS22647
CVI-0	CS22614	Mrk-0	CS22635	Ts-5	CS22648
Eden-1	CS22572	Ms-0	CS22655	Tsu-1	CS22641
Eden-2	CS22573	Mt-0	CS22642	UII2-3	CS22587
Edi-0	CS22657	Mz-0	CS22636	UII2-5	CS22586
Ei-2	CS22616	Nd-1	CS22619	Uod-1	CS22612
Est-1	CS22629	NFA-10	CS22599	Uod-7	CS22613
Fab-2	CS22576	NFA-8	CS22598	Van-0	CS22627
Fab-4	CS22577	Nok-3	CS22643	Vår2-1	CS22580
Fei-0	CS22645	ÖMö2-1	CS22584	Vår2-6	CS22581
Ga-0	CS22634	ÖMö2-3	CS22585	Wa-1	CS22644
Got-22	CS22609	Oy-0	CS22658	Wei-0	CS22622
Got-7	CS22608	Pna-17	CS22570	Ws-0	CS22623
Gu-0	CS22617	Pro-0	CS22649	Ws-2	CS22659
Gy-0	CS22631	Pu2-23	CS22593	Wt-5	CS22637
HR-10	CS22597	Pu2-7	CS22592	Yo-0	CS22624
HR-5	CS22596	Ra-0	CS22632	Zdr-1	CS22588
Kas-2	CS6751	Ren-1	CS22610	Zdr-6	CS22589

**Table S1. *A. thaliana* accessions phenotyped for GWA.**

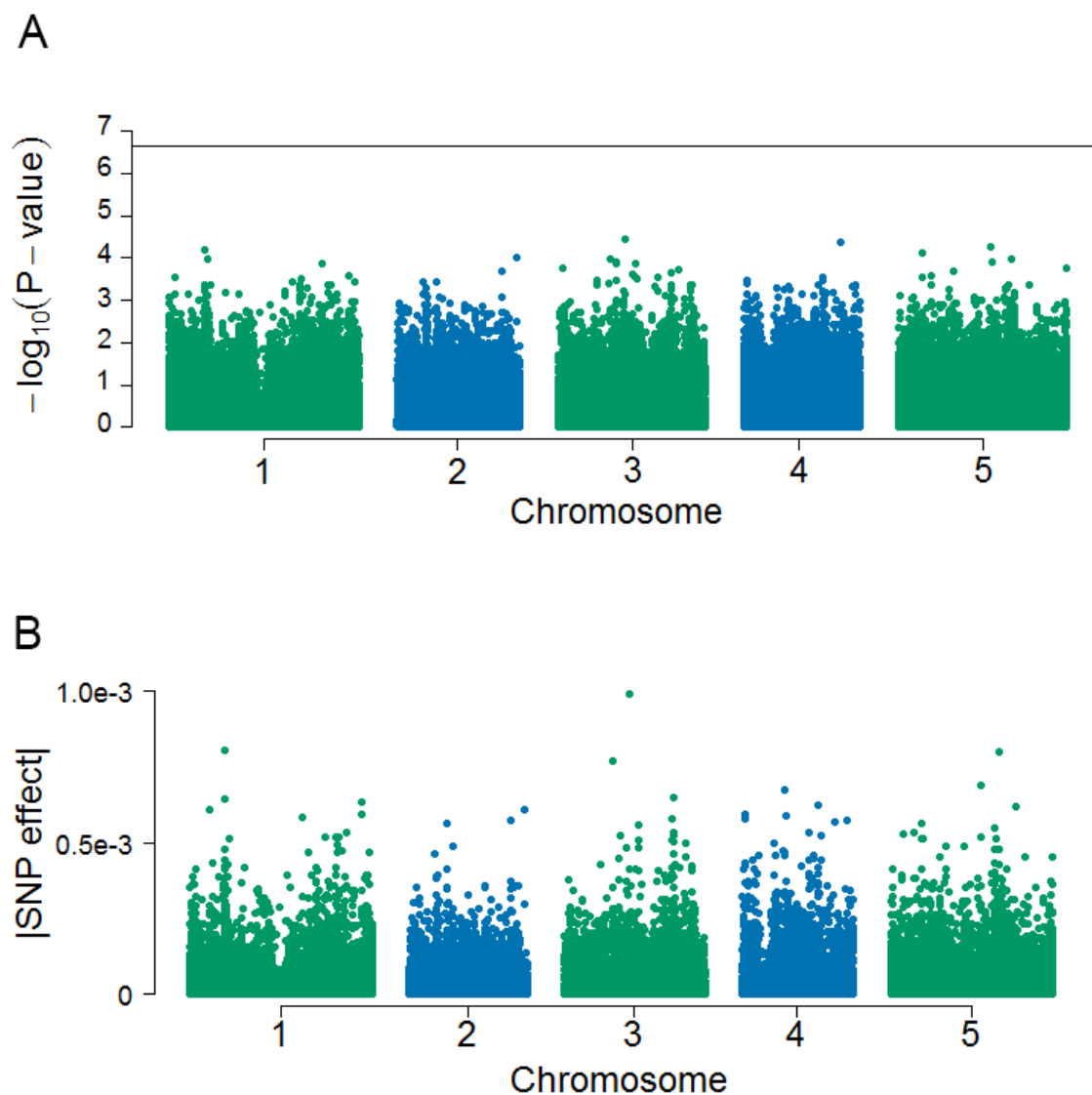
A



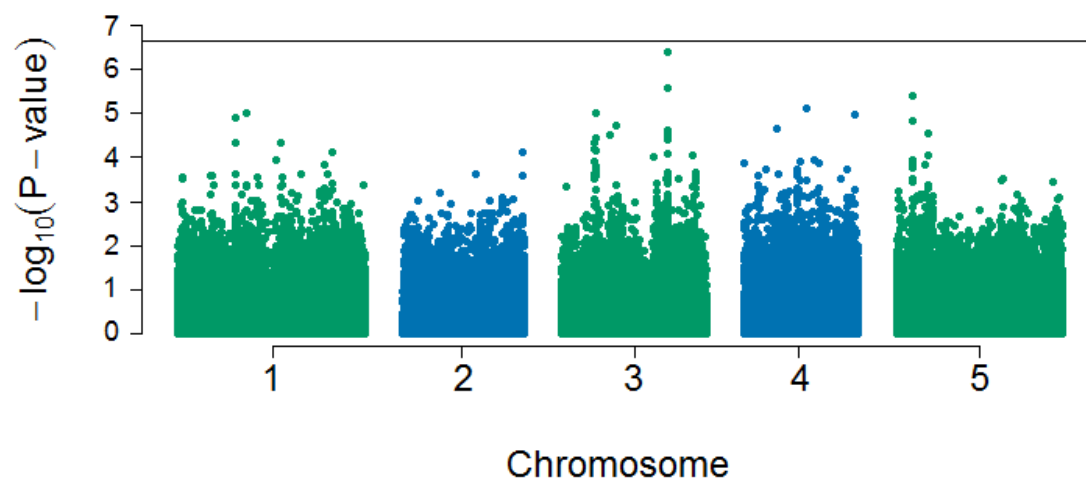
B



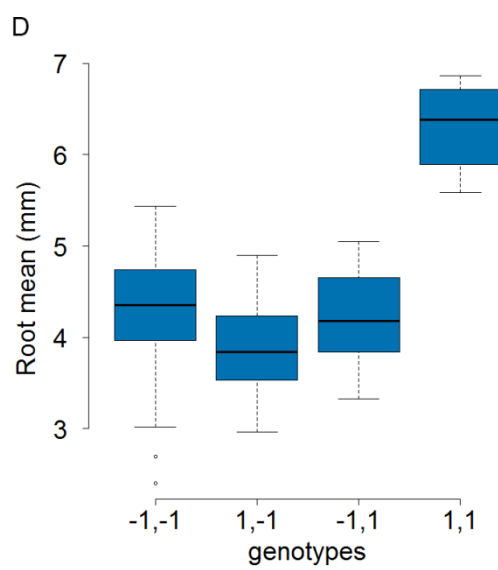
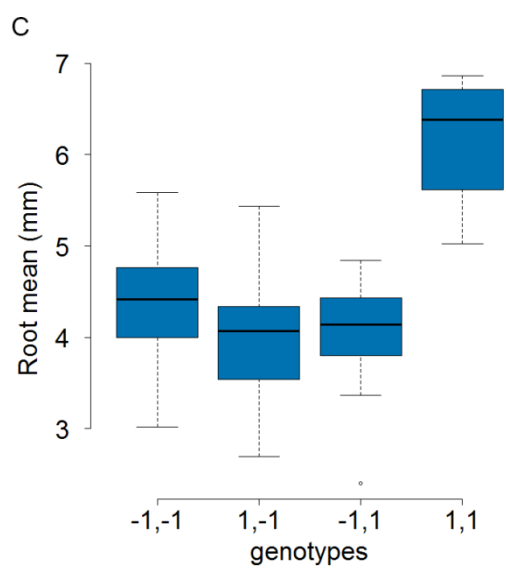
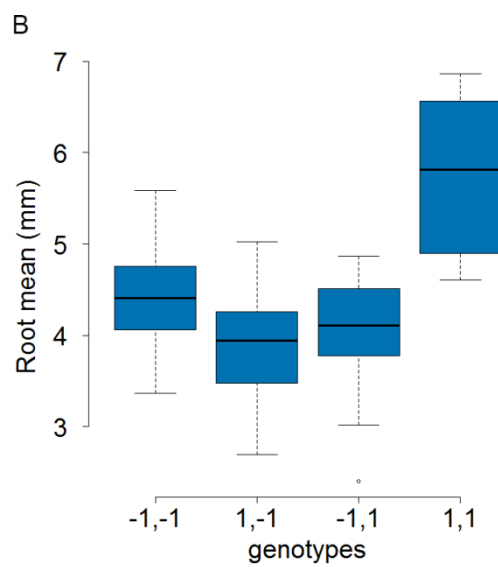
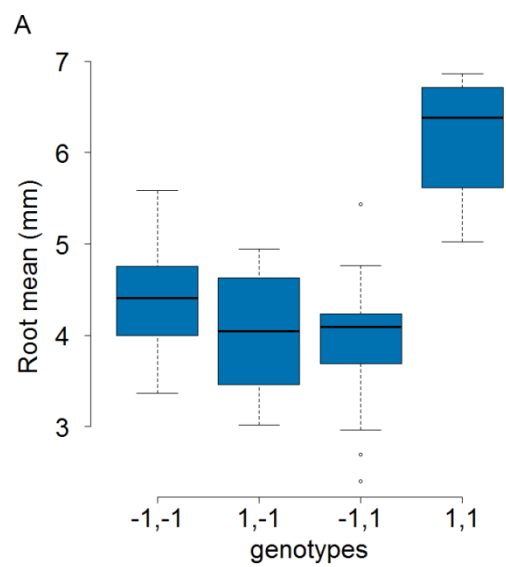
**Figure S2. Root length variance varies independently of root mean and can be calculated globally or per replicate.** A) There is a strong correlation between the total variance estimated from ~210 individuals and mean variance from three replicates of ~70 individuals ( $p < 2.2e-16$ ,  $R^2 = 0.98$ ). B) There is no significant correlation between root length mean and root length variance ( $p = 0.11$ ,  $R^2 = 0.16$ , Pearson's).



**Figure S3. No associations are found with root length using an additive model.** A) A GWAS was completed under an additive model. No SNPs reached the Bonferroni threshold. B) Using a whole-genome generalized ridge regression model in which SNPs were random effects, no SNPs reached the Bonferroni threshold.



**Figure S4. No loci are detected for root length variance using GWAS with an additive model.** A GWAS was completed under an additive model. No SNPs reached the Bonferroni threshold.



**Figure S5. The same pattern of effects is observed for all significantly interacting loci. A-D)**

Root mean is displayed for the four pairs of significantly interacting loci. The major allele is indicated by -1 and the minor allele is indicated by 1. The combination of two minor alleles (1,1) always has the largest root length compared to the other three allele combinations, which are more similar to one another. A) Root mean for allele combinations at 1\_17257526 and 5\_15862026 / 5\_15862525. B) Root mean for allele combinations at 3\_66596 and 3\_9272294 / 3\_9273674. C) Root mean for allele combination at 3\_66596 and 5\_18241640. D) Root mean for allele combination at 3\_10891195 and 5\_1027939.

Gene	T-DNA line	Insertion location
At3g01185	SALK_033165	promoter
At3g01200	SALK_035653	Exon 1 of 3
At3g25520	SALK_135037	5'UTR
At3g25530	SALK_057410	Intron 3 of 7
At3g25540	SALK_073240	Exon 1 of 5
At5g45120	SALK_030666	5'UTR
At5g39610	SALK_090154	Exon 3 of 3
At5g39620	SALK_096950	Intergenic
At3g28865	SALK_089352	Exon 1 of 1
At3g28865	SALK_009759	Exon 1 of 1
At3g28880	SALK_070443	Exon 17 of 19
At5g03840	SALK_142051	5' UTR
At5g03850	SALK_112775	Exon 1 of 1

**Table S2. T-DNA lines tested for root phenotypes.**